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GRANT NUMBER DAMD17-94-J-4382

TITLE: Human Breast Cancer Cell/Tissue Bank and Database

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REPORT DATE: September 1996

TYPE OF REPORT: Annual

PREPARED FOR: Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	September 1996	Annual (1 Sep 95 - 31 Aug 96)

4. TITLE AND SUBTITLE
Human Breast Cancer Cell/Tissue Bank and Database

5. FUNDING NUMBERS
DAMD17-94-J-4382

6. AUTHOR(S)
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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, MD 21702-5012

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200*

The second year of the University of Michigan Human Breast Cell/Tissue Bank and Data base has been dedicated to advertising the existence of this resource and providing investigators from around the country and internationally with the cells and tissues we have banked. A World Wide Web page for advertising this resource was developed and put on-line. This web site contains an on-line order form that allows investigators to request, via e-mail, the cells or tissues that suits their needs. During the past year, this web site has been visited over 1000 times and many investigators have made use of the on-line order form requests for breast cancer cells and tissues. Thus, over the past year, resource has distributed frozen human breast cancer cells, breast cancer cell lines, and paraffin embedded and frozen histologic sections from breast cancer specimens. Goals for the upcoming year are to improve our data base so that clinical, histopathologic and immunohistochemical data are readily available to investigators who get cells and tissues from this resource, and to improve the visibility of this resource in order to increase the number of requests that we receive and fill.

14. SUBJECT TERMS	15. NUMBER OF PAGES
Breast Cancer, Tissue Bank, Data Base, Humans, Anatomical	21
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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Stephen P. Ft 9-26-96

PI - Signature Date

Stephen Ethier, Ph.D.

Human Breast Cancer Cell / Tissue Bank and Database

Table of Contents

INTRODUCTION

The major goal of the work that is supported by this DOD infrastructure grant is to develop a human breast cancer cell/tissue bank and data base to facilitate distribution of fresh breast cancer specimens to breast cancer researchers in our own institution and throughout the country. Over the past year, we have implemented many of the plans and infrastucture that was developed during year one and have begun to disburse human breast cancer cells and tissues to investigators nation wide. Many aspects of our resource are unique. For example, we are the only such tissue bank that provides both normal and neoplastic breast epithelial cells in a viable state suitable for in vitro studies as well as for the more common molecular biological applications. We are providing frozen sections and touch preps in addition to the more common paraffin embedded sections. We are providing clinical data on each patient sample that includes the common clinical information, such as estrogen and progesterone receptor statues and lymph node status. We are also now providing data on expression of certain oncogenes and tumor suppresser genes (erbB-2 and P53 expression status) in these samples. Finally, we are actively establishing new human breast cancer cell lines which we provide to the scientific community. Our cell lines are cultured under growth factor-defined conditions and come with a full array of cellular and molecular data. Furthermore, cells from these lines are provided at early passage levels, thus, providing a better model of breast cancer cell growth in vivo. To data, we have established 10 new human breast cancer cell lines from early and late stage primary tumors and from metastatic sites.

Thus, we have developed a unique human breast cell/tissue bank and data base that is now providing novel and important cells and tissues to many breast cancer researchers.

BODY

During the past year, much work has been done to address and complete the tasks that were originally laid out in the Statement of Work. Therefore, this report will comment on the progress as it relates to each of those tasks.

Task 1. Establish logistic methods for insuring that every human breast cancer specimen that has tissue remaining following evaluation by the Surgical Pathologist is routed to the Cancer Biology laboratory for tissue preparation, cell isolation and cyropreservation in the cell bank.

Most of the work relevant to this task was accomplished during year one of the grant. Thus, by the beginning of year 2, the mechanisms were already in place to insure that all new human breast cancer specimens became part of our growing cell and tissue resource. A tissue procurement technician now routinely obtains all biospied breast cancer material from the surgical pathology suite of the operating room, and with the approval of a staff pathologist, obtains any non-used tissue and directs it to the cell biology laboratory for breast cancer cell

isolation, banking and cell line development. For tumors of sufficient size, solid pieces are frozen directly in liquid nitrogen for the preparation of touch preps and frozen sections. Finally, all paraffin embedded breast cancers are included in the bank of tumors available for the preparation of new histologic sections.

At the present time, there are 269 breast cancer specimens banked as paraffin embedded tissues, for which clinical, histopathologic and immunohistochemical data are available. The frozen bank of isolated human breast cancer cells now consists of over 800 ampoules of cells from 126 different patients. Also, during the past year, new human breast cancer cell lines have been established, bringing the total developed in this laboratory to ten. All of these lines are part of this resource and many of these lines have been distributed to investigators.

Task 2. Establish methods for histologic evaluation of parameters not ordinarily evaluated for human breast cancer specimens, for all specimens that yield cells that are stored in the tissue bank. These include immunohistochemical evaluation of expression levels of p53 protein, EGFR receptor and HER-2/neu receptor. Progress toward this task was discussed briefly above. In addition to routine histopathologic analysis done on all breast cancer specimens, all breast cancer specimens that are part of our cell/tissue bank are now evaluated separately for expression of erbB-2 and p53. As mentioned above, we now have 269 banked breast cancer specimens with data on erbB-2 and p53, in addition to the routine histopathologic information.

Task 3. Establish logistic methods for routine blood drawing of all breast cancer patients whose cells are preserved in the bank, in order to isolate and immortalize lymphocytes from these patients. In last years report, we alluded to some logistic difficulties with obtaining immortalized lymphocytes for our patient samples. Although some of those logistic problems remain, so far, there has been only one request for immortalized lymphocytes. It turns out that obtaining immortalized lymphocytes on an ad hoc basis on those rare occasions when those cells are requested is straight forward and we were, indeed, able to obtain immortalized lymphocytes from one patient this year. Unless the level of interest in this aspect of our bank increases dramatically we will continue to obtain these cells only on an "as needed" basis and we will continue to make use of the medical center core facility that is set up to immortalize human lymphocytes using EBV.

Task 4. Establish a computerized data base for all patients whose cells are currently stored in the cell bank and for all future patients whose cells/tissues are banked. The data base will contain all pertinent family history data, all data obtained from histopathologic evaluation of the breast cancer specimen, the location and status of the patients' cells and tissues stored in the

bank. Experimental data obtained by individual investigators using banked samples will also be entered into the data base. The data base will be set-up in a way that allows investigators to access patient data without compromising the privacy and confidentiality of the patient.

The current data base is divided into sections that contain; demographic data for all patients whose cells and tissues are banked, pathological data for all specimens, data on the availability of fresh cells, and family history data for patients whose cells and tissues have been banked. The various parts of the data base are connected in ways that allow multiparameter searches of our entire data base. This allows us to provide the kinds of specimens that investigators request for specific subsets of patient samples. During year two, many technical problems were solved such that we now have a fully operational data base that contains all of the data in one central location. This turned out to be more difficult that previously envisioned in that the separate clinical, pathology and cell biology data bases were administered by different persons and were kept in different formats. Thus, merging them into one centralized data base was a task that was worked on for most of this year and is now complete.

During the construction of this data base, many precautions were taken to protect the identity of patients whose cells/tissues are banked. First, the server that contains the data base is heavily password protected. In addition, each patient sample in the bank is assigned a unique number, referred to as a Key ID, that cannot be linked to the patients' identity. Thus, any clinical data that is provided with the specimen is only linked to that unique number.

The data base, as it now stands, is a powerful supplement to the cell/tissue resource and allows us to fulfill specific needs of many breast cancer researchers. Recently, we hired a new data manager that is supported by this grant, who's major task is to integrate all of the data on the specimens in our bank and keep the central data base up-to-date. In addition, all requests for cells and tissues are routed to this data manager who keeps the data base current, and is also responsible for obtaining appropriate data that is given to investigators along with the cells or tissues they receive. Thus, the data base is now fully prepared to find specimens within our growing bank of cells and tissues that suit the needs of specific investigators. For example, we recently received a request for cells from stage II breast cancers that overexpressed erbB-2. By combining the histopathologic data and the immunohistochemical data, we were able to identify frozen cells within the bank from tumors that met these criteria. Thus, the data base is organized and operational and well positioned for the next phase of the development of our resource.

Task 5. Set up and implement the administrative plan for distribution of cells and tissues stored in the bank to other investigators at the University of Michigan as well as other Cancer Centers throughout the mid-west and the country. During the first year, we developed an ordering procedure for the distribution of cells and tissues in the bank. The order form that was

developed sets limits on availability of various specimen types. In addition, we have set up priority criteria that will drive decisions to disburse cells/tissues in the event that availability of particular specimens becomes limited.

During year two, we set out to advertise more widely the availability of cells and tissues from this resource. We did this in two ways. First, handouts were prepared and distributed at breast cancer scientific meetings attended by the P.I. during 1995. These handouts met with enthusiastic response. Second, a world wide web site was constructed and put on line during year two. This web site contains a menu of cells and tissues available from the resource as well as an on-line request form. Investigators can fill out the form on line, and by clicking on the hypertext "send" can route the order to the P.I. via e-mail. Recently, the web site was linked to other breast cancer sites and data bases. The URL for our web site is:

http://www.cancer.med.umich.edu/umbnkdb.html

These approaches have resulted in our receiving several requests for cells and tissues during this year. To date, the web site has been visited over 1000 times. We have received 29 requests for cells and tissues during year 2.

Nine of these requests were directed to the Pathology group. To fill these requests the Pathology group reviewed 90 cases, and prepared 163 haematoxylin and eosin stained sections from paraffin embedded tissues and prepared 84 frozen sections. In addition, six frozen pieces of human breast cancer cells were distributed by the Pathology group.

A total of 20 requests for cells and cell lines came to the Cell Biology group during the past year. To fill these requests, 21 ampoules of frozen breast cancer cells, 83 ampoules of breast cancer cell lines, and 4 ampoules of normal human mammary epithelial cells were distributed to various investigators.

A series of tables are presented in the appendix which give details on the specimens disbursed from our resource during year 2 of the grant, as well as a list of the investigators who have received breast cancer cells and tissues from us.

CONCLUSIONS

In summary, the second year of this project has been quite successful with much progress made toward all of the specific tasks outlined in the Statement of Work. We feel strongly that the cell and tissue bank that we have developed provides many unique features to breast cancer researchers and will make a strong contribution to many areas of breast cancer research. The main goal for the next year of this project is to increase the visibility of this resource so as to increase significantly the number of requests for cells and tissues that we receive and fill. This will be done by placing ads in selected scientific journals and by direct mailings to individuals whose focus is breast cancer research. The infrastructure is clearly in place and we have

demonstrated that we can provide breast cancer cells and tissues to investigators. The next challenge is broaden the scope of this resource in order to provide these cells and tissues to all breast cancer researchers that can benefit from the special aspects of our cell and tissue bank.

REFERENCES

none.

APPENDIX

1995 - 1996 Database Report

Total Number of Patients in the Database = 269

Pathology

There were **nine requests** from Principal Investigators working within the United States (CA: 2; MA: 1; MI: 5; VA: 1)

From the nine requests made to Pathology, **ninety cases were reviewed.**
The following breakdown is what was sent to those nine Investigators:

Requests fulfilled by Pathology 1995-96

Paraffin:
163 Haematoxylin & Eosin (H/E)
448 Histologic glass slides (blanks)
60 silane slides

Frozen:
84 Frozen sections
6 Frozen tissue chunks

Requests fulfilled by Pathology 1995-96

Date Fulfilled	# Cases	PETS	Total Slides
09/08/95	10 Ca & 10 NI	5 blank & 1 H/E	120
10/24/95	15 Ca &15 NI	3 blank & 1 H/E	120
10/24/95	12 Ca & 12 NI	3 blank & 1 H/E	96
12/26/95	15 Ca & 15 NI	3 blank & 1 H/E	120
03/06/96	3 frozen chunks (2@)		6 frozen chunks
03/06/96	7 Ca	12 frozen of each	84 frozen sections
		1 H/E of Ca	7
		3 blank & 1 H/E NI	28
03/22/96	10 Ca & 10 NI	3 silane & 1 H/E	60 silane / 20
04/10/96	11 Ca & 11 NI	3 blank & 1 H/E	88
	16 Ca & NI	3 blank & 1 H/E	128
04/11/96	6 Ca	3 blank & 1 H/E	24
	7 Ca & 7 NI	3 blank & 1 H/E	56
05/22/96	5 NI	3 blank & 1 H/E	20
			Total Slides: 887
			Total Frozen Chunks: 6
			Total Frozen Sections: 84

There were no Touch preps requested or given.
All of the above data was accompanied by Pathological Data.

Laurie Robyn Blumberg

Cancer Biology

From October 1995 to the present, there has been an increase of:
135 Frozen segments from Breast Cancer patients and 56 Human Mammary Epithelial (HME's).

Number of Frozen segments and HME's 1995-96

Frozen segments:		Human Mammary Epithelial:		**Total**
October 1995:	2	October 1995:	9	11
November:	16	November:	0	16
December:	8	December:	0	8
January 1996:	0	January 1996:	0	0
February:	0	February:	0	0
March:	3	March:	11	14
April:	32	April:	1	33
May:	3	May:	8	11
June:	50	June:	27	77
July:	15	July:	0	15
August:	4	August:	0	4
September:	2	September:	0	2
Total	135		56	191

There were **twenty requests** from individual Principal Investigators
(CA: 2; MD: 5; MI: 3; NY: 5; PA: 2; TX: 1; VA: 1; and Israel: 1)

From the twenty requests made, **108 Samples** were distributed.

Requests Fulfilled by Cancer Biology 1995-1996

Frozen Cancer Cells, Cell lines, and HME's Distributed

Date	Frozen Cells	Cell lines	Normal Cells	**Total by month**
Oct-95	10		2	12
Nov-95				
Dec-95				
Jan-96				
Feb-96		15		15
Mar-96	4			4
Apr-96		16		16
May-96	3	9	2	14
Jun-96	4	22		26
Jul-96		7		7
Aug-96		12		12
Sep-96		2		2
Total of each item	21	83	4	**108**

Distribution of Cell lines

Date	SUM-44PE	SUM-52PE	SUM102PT	SUM-149PT	SUM-1315MO2	SUM-159PT	Total
Oct-95							
Sep-95							
Oct-95							
Nov-95							
Dec-95							
Jan-96							
Feb-96	4	4	4	1	1	1	15
Mar-96							
Apr-96	4	4			4	4	16
May-96	1	2	3	1	1	1	9
Jun-96	3	7	3	3	3	3	22
Jul-96	1	2	2	1		1	7
Aug-96	2	2	2	2	2	2	12
Sep-96			1	1			2
Total	15	21	15	9	11	12	**83**

Sheet1

Principal Investigators Requesting Data			
Name	Department	Institution	City/State/Country
Aldaz, Marcelo		U. of Texas MD Anderson Cancer Ct	Houston, Texas
Asch, Bonnie		Roswell Park Cancer Institute	Buffalo, NY.
Cohen, Stanley N.	Dept. of Genetics	Stanford Medical Center	Santa Cruz, CA.
Cohen, Stanley N.	Dept. of Genetics	Stanford Medical Center	Santa Cruz, CA.
Couch, Fergus		U. of Penn Medical Center	Phila., PA.
Gabrielson, Edward		Johns Hopkins Pathology Research	Baltimore, MD.
Gelman, Irwin		Mt. Sinai School of Medicine	New York City, NY.
Gelman, Irwin		Mt. Sinai School of Medicine	New York City, NY.
Gottardis, Marco		Ligand Pharmaceuticals	San Diego, CA.
Kurnit, David	Dept. of Pediatrics	University of Michigan	Ann Arbor, MI.
Merajver, Sofia	Dept of Hem/Onc.	University of Michigan	Ann Arbor, MI.
Merajver, Sofia	Dept of Hem/Onc.	University of Michigan	Ann Arbor, MI.
Parsons, Sarah J.	Dept. of Microbiology	UVA Health Science Center	Charloettesville, VA.
Parsons, Sarah J.	Dept. of Microbiology	UVA Health Science Center	Charloettesville, VA.
Petty, Liz	Dept. of Internal Med.	University of Michigan	Ann Arbor, MI.
Petty, Liz	Dept. of Internal Med.	University of Michigan	Ann Arbor, MI.
Petty, Liz	Dept. of Internal Med.	University of Michigan	Ann Arbor, MI.
Petty, Liz	Dept. of Internal Med.	University of Michigan	Ann Arbor, MI.
Rotenberg, Susan		Queens College, CUNY	New York
Ryan, Patricia		Genetic Therapy	Gaithersburg, MD.
Ryan, Patricia		Genetic Therapy	Gaithersburg, MD.
Ryan, Patricia		Genetic Therapy	Gaithersburg, MD.
Salomon, David		NCI, NIH	Bethesda, MD.
Silverman, Gary		Childrens' Hospital	Boston, MA.
Silverstein, Gary	Dept. of Biology	Sinsheimer Laboratories (U of CA.)	Santa Cruz, CA.
Strayer, David		Thomas Jefferson Medical College	Phila., PA.
Welsh, JoEllen		Alton Jones Cell Science Center	Lake Placid, NY.
Wicha, Max		Univ. of Michigan	Ann Arbor, MI.
Yarden, Yosef		Weizman Institute of Science	Rehovot, Israel
Yu, Ben		Henry Ford Health System	Michigan

The following pages are from the World Wide Web Site for

The University of Michigan Human Breast Cell/Tissue Bank and Data Base

The University Of Michigan Human Breast Cell/Tissue Bank And Data Base

Stephen P. Ethier, Ph.D., Sofia Merajver, M.D., Ph.D., and Tom Giordano, M.D., Ph.D.
Departments of Radiation Oncology, Internal Medicine and Pathology, The University of Michigan Medical School, Ann Arbor, MI 48109

In 1995 **The University of Michigan Breast Cell/Tissue Bank and Data Base** came into existence as a result of an infrastructure grant awarded to the U of M by the Department of Defense. This resource had been developed internally over the previous six years as a result of the Principal Investigators interest in breast cancer biology and because of the need to carry out breast cancer biology studies using freshly isolated human breast cancer cells. The need for new human breast cancer cell lines that can be cultured under defined conditions in vitro, and for which both patient information and molecular data are available, also contributed to the motivation to develop this resource.

The purpose of this resource is to provide breast cancer researchers with primary breast cells and tissues in a variety of forms that are suitable for a variety of experimental approaches. Breast cells and tissues obtained from this bank come with both demographic and clinical data on the patient sample. Furthermore, users may request cell/tissue samples from patients with specific characteristics, e.g., ER positive or P53 positive cells.

The "menu" given below provides a brief description of what is currently available in this resource. An order form can be found after the menu, and cell/tissue requests can be made on line. Questions regarding this resource can be e-mailed to Dr. Ethier at: spethier@umich.edu

UM-HUMAN BREAST CANCER CELL/TISSUE BANK AND DATA BASE MENU:

1. BREAST TISSUE

A. Histologic sections from matched neoplastic and nonneoplastic paraffin embedded tissues: These specimens are useful for morphologic evaluation, immunohistochemistry, and DNA extraction.

B. Frozen histologic sections. These specimens can be used for immunohistochemistry and for isolation of DNA or RNA. In addition, limited samples of snap frozen neoplastic and nonneoplastic breast cancers are available for biochemical and molecular studies.

C. Touch preps from freshly isolated breast cancer specimens: Limited numbers of these specimens are available prospectively and are useful for immunohistochemistry, in situ hybridization and fluorescence in situ hybridization (FISH).

D. Frozen human breast cancer cells isolated from primary or metastatic sites: Primary human breast cancer specimens are enzymatically dissociated and breast cancer cells are isolated and frozen immediately at -150 degrees. Typically, ampoules consist of 10 million cells that represent a mixture of neoplastic breast epithelial cells, normal breast epithelial cells and stromal cells. The current cell bank consists of over 400 ampoules of frozen cells obtained from over 80 different patients. These samples are useful for tissue culture studies of breast cancer cells, normal breast epithelial cells and breast stromal cells. These cells can also be used for isolation of DNA, RNA and protein for biochemical and molecular analyses.

E. Frozen normal breast epithelial cells obtained from reduction mammoplasty specimens: A limited number of specimens are currently available and can be used for tissue culture of normal mammary cell components and for isolation of DNA, RNA and protein.

F. Human breast cancer cell lines: At present six human breast cancer cell lines have been developed from specimens in our cell bank. All of these cell lines were isolated under, and have been continually maintained in, growth factor defined conditions. These cell lines have been characterized for their growth factor requirements and are currently being characterized for molecular alterations known to occur in human breast cancer. Early passage versions of all of these lines are available. Please refer to the SUM-breast cancer cell line page for more information on the individual cell lines.

2. IMMORTALIZED LYMPHOCYTES

Blood is collected from selected patients whose cells are stored in our bank. There is a limited ability to provide matched immortalized lymphocytes from patients whose cells/tissues are banked. These immortalized lymphocytes provide a source of normal DNA to be used in conjunction with breast cancer cells and tissues obtained from matched patients.

3. CLINICAL AND PATHOLOGIC DATA BASE

A data base has been developed to accompany the cell and tissue resource. The goal of this data base is to provide clinical and pathologic parameters, if needed, on cells or tissue samples that investigators obtain from the bank. The following information is available from this data base:
-demographic data
-family history data
-histopathologic diagnosis of tumor
-tumor size, grade and stage
-lymph node status
-estrogen/progesterone receptor status
-p53 and HER2/neu expression status by immunohistochemistry

If requested, this information will be provided to investigators in a coded fashion that does not allow linkage to the patients identity and does not compromise patient confidentiality.

You may request breast cancer Cells, tissue sections and data via this Order Form

OTHER BREAST CELL BANKS AND DATABASES

Human Mammary Epithelial Cells (HMEC) NEW

Request for Breast Tissue/Clinical Data Bank

Note: This is an HTML form. Some WWW browsers might not support it. If you can't use this form, please send e-mail to spethier@umich.edu with the following information.

Please make sure that all the fields have been filled before you send the order.

Principal Investigator: Phone #: (000)-000-0000

Contact Person (if different): none

Academic Title: none e-mail address:

Mailing Address: Fax #: (000)-000-0000

Project Title:

none

Funding:

Tissue Requested: (check appropriate butt

Sample type request

1) Breast Tissue
   a) Histologic Paraffin Sections
      - neoplastic only.....
      - matched non-neoplastic/neoplastic.....
   b) Touch Preps (3-5 per sample, as available).....
   c) Frozen Tissue Sections (6 slides/sample).....
   d) Frozen Breast Cancer Cells.....
   e) Frozen Normal Breast Epithelial cells.....
   f) Human Breast Cancer Cell Lines .....
   g) Other (specify below).....

   h) Please enter the Number of samples requests : 0

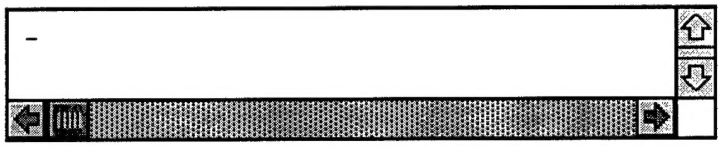
2) Clinical Data Base — Clinical data required for specimens obtained

◉ yes ○ no

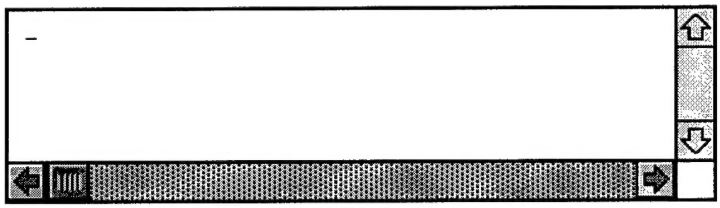
3) Pathology Data Base — Pathologic data required for specimens obtain

◉ yes ○ no

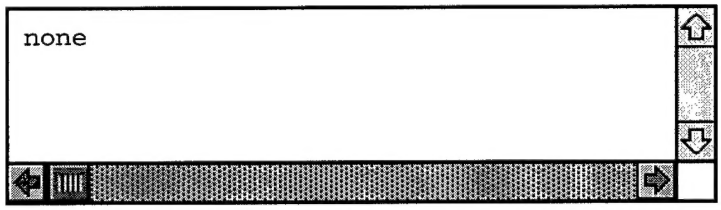
If you selected "Other" above, please specify the type of cell tissue needed.



Briefly describe project, including needs for and uses of tissue and database. Please justify use of requested number of samples.



Describe any special patient characteristics or limiting characteristics (e.g., age, menopausal status, etc.)



Click [Send Request] To Mail Your Request.

Click [Clear Request Form] to Clear This Form and Start Over.

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Human Breast Cancer Cell Lines (SUM-LINES)

SUM-44PE was developed from a pleural effusion from a patient with ER positive breast cancer and has been in culture for over two years.

SUM-52PE was developed from a pleural effusion from a patient with ER negative breast cancer. These cells have been in culture continuously for 1.5 years.

SUM-102PT was developed from a primary infiltrating ductal carcinoma of the breast following completion of neo-adjuvant chemotherapy. These cells have been in culture for over one year.

SUM-149PT is a recently developed cell line from a primary infiltrating ductal carcinima of the breast from a patient with locally advanced disease.

SUM-1315MO2 is a cell line developed from a mouse xenograft derived by transplantation of a metastatic nodule of a patient with infiltrating ductal carcinoma.

SUM-159PT is a cell line isolated from a primary tumor of a patient with metaplastic carcinoma of the breast.

All of the cell lines described above are karyotypically abnormal, cytokeratin positive, and express breast epithelial antigens.

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SUM 52

Cell Source	Pleural Effusion
ER/PR Status	Er-/PR-
Lymph Node Status	
Culture Media	Ham's F-12 with 5% Fetal Bovine Serum, Insulin & Hydrocortisone added; Serum-Free Ham's F-12 with Insulin & Hydrocortisone added
Oncogene amplification	FGFR-1, FGFR-2
Growth Factor Receptor Express (+ by RT-PCR)	Her-2, Her-3, Her-4, IGFR-1, InR, FGFR-1, FGFR-2
p53 (SSCP)	Deletion of Exons 6-10
p53 (staining)	Negative
pRb status (Western Blot)	Positive
TGF-beta response	Resistant
Bcl-2 status	Negative
Bcl-x status	Positive

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